

Interaction of aerobic soil bacteria with plutonium(VI)

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Summary. We studied the interaction of Pu(VI) with *Pseudomonas stutzeri* ATCC 17588 and *Bacillus sphaericus* ATCC 14577, representatives of the main aerobic groups of soil bacteria present in the upper soil layers. The biosorption studies have shown that these soil bacteria accumulate high amounts of Pu(VI). The relative sorption efficiency toward Pu(VI) related to the amount of biomass used decreased with increasing biomass concentration due to increased agglomeration of the bacteria resulting in a decrease of the number of available complexing groups. Spores of *Bacillus sphaericus* showed a higher biosorption than the vegetative cells at low biomass concentration which decreased significantly with increasing biomass concentration. At higher biomass concentrations (> 0.7 g/L), the vegetative cells of both strains and the spores of *B. sphaericus* showed comparable sorption efficiencies. Investigations on the pH dependency of the biosorption and extraction studies with 0.01 M EDTA solution have shown that the biosorption of plutonium is a reversible process and the plutonium is bound by surface complexation. Optical absorption spectroscopy showed that one third of the initially present Pu(VI) was reduced to Pu(V) after 24 hours. Kinetic studies and solvent extraction to separate different oxidation states of Pu after contact with the biomass provided further information on the yield and the kinetics of the bacteria-mediated reduction. Long-term studies showed that also 16% of Pu(IV) was formed after one month. The slow kinetics of this process indicate that under our experimental conditions the Pu(IV) was not a product by microbial reduction but seemed to be rather the result of the disproportionation of the formed Pu(V) or autoreduction of Pu(VI).

Introduction

The production and testing of nuclear weapons, nuclear reactor accidents and accidents during the transport of nuclear weapons have caused significant environmental contamination with radionuclides. Their migration behavior is controlled by a variety of complex chemical and geochemical reactions such as solubility, hydrolysis, redox reactions and complexation reactions with inorganic, organic and biological ligands, and sorption on the geo-matrix. In addition, microorganisms can strongly influence the actinides'

transport behavior by both direct interaction (biosorption, bioaccumulation, oxidation and reduction reactions) and indirect interaction (change of pH and redox potential), thus immobilizing or mobilizing the radionuclides. Especially plutonium is a cause for major concern due to the quantity in nuclear waste, the toxicity and the long half lives of the different isotopes. Fundamental information on the mechanism of biotransformation of plutonium will be useful in predicting the microbial impact on the migration from waste repositories as well as developing remediation and decontamination strategies for contaminated sites.

Plutonium can exhibit several oxidation states, e.g., 4+, 5+, and 6+, in aqueous solution under environmental conditions [1]. Most papers on the interaction of plutonium with bacteria contain only data on the amount taken up by the bacteria [2–4] without providing information on mechanistic details of the interactions with the biomass. Due to the fact that plutonium is a redox active metal, the interaction with the biomass can cause changes of the oxidation state. For a better understanding of these processes, a speciation of the plutonium complexes formed with the bacteria is necessary. In recent years, a first attempt was made to characterize the reduction products of Pu(IV) after contact with iron-reducing bacteria [5]. The authors observed an increased solubilization of hydrous PuO₂(s) under anaerobic conditions in presence of the bacteria. Unfortunately, the predicted formation of Pu(III) could not be proven. The solutions contained nitrilotriacetic acid (NTA) and, thus, the Pu(III) was immediately oxidized to Pu(IV). This is in good agreement with literature data on the stability of various plutonium valence states in the presence of NTA [6].

Our research focuses on the interaction of aerobic bacteria with hexavalent plutonium. Because of the high solubility compared to Pu(IV) and the stability under aerobic conditions, plutonium(VI) has a high potential of migration away from waste repositories and, thus, for contamination of the environment. We used two different strains for our studies, *Bacillus sphaericus* (ATCC 14577) and *Pseudomonas stutzeri* (ATCC 17588), representatives of the main aerobic groups of soil bacteria. Our studies included biosorption studies to determine the sorption efficiency toward plutonium under various conditions (pH, amount of biomass) and desorption studies to obtain information on the nature (sorption, surface complexation, or uptake) and binding strength of the interaction. To characterize the oxidation states of the

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formed plutonium species after contact with the bacteria, we used solvent extraction techniques and optical absorption spectroscopy.

Experimental

The bacterial strains (*Bacillus sphaericus* ATCC 14577 and *Pseudomonas stutzeri* ATCC 17588) were grown under aerobic conditions in 500 mL nutrient medium (8 g/L nutrient broth, Difco) at 22 °C. The biomass was separated from the growth medium by centrifugation (6000 × g) and washed 4 times each with 40 mL physiological NaCl-solution (0.9%). To study the sorption efficiency of *B. sphaericus* in dependency of the age of the culture, a 24-hour culture containing fresh vegetative cells, a 27-hour culture with vegetative cells before they started to sporulate, and a culture of spores (48 hours) without vegetative cells were prepared.

A ^{239}Pu stock solution was prepared by ion exchange chromatography purification (Dowex AG1-X8 anion resin) and oxidation to Pu(VI) with concentrated HClO_4 . The stock solution was diluted to 20 mL containing 8.6 g/L of Pu(VI). The purity of the oxidation state was verified by absorption spectroscopy.

To study biosorption, we used a Pu(VI) concentration of 36.7 mg/L in 1.5 mL 0.9% NaCl-solution at pH 5. We varied the biomass from 0.08 g_{dry weight}/L to 1.29 g_{dry weight}/L (*Pseudomonas stutzeri*) and from 0.07 g_{dry weight}/L to 1.17 g_{dry weight}/L (*Bacillus sphaericus*, vegetative cells) and 0.07 g_{dry weight}/L to 1.15 g_{dry weight}/L (*Bacillus sphaericus*, spores). To obtain information on the binding strength and the reversibility of the biosorption process, the plutonium on the biomass was extracted with 0.01 M EDTA solution (pH 5). Plutonium concentrations were measured by liquid scintillation counting using a LKB Wallac, 1219 Rackbeta liquid scintillation counter.

In order to obtain information on the Pu species involved, the oxidation states formed and the time scale of the biosorption processes, optical absorption spectroscopy was used. The spectroscopic studies were performed in 5 mL polyethylene (PE) cuvettes, using Pu(VI) concentrations between 85.6 and 127.0 mg/L in 2 mL physiological NaCl-solution. The biomass was added in 20 µL portions of the bacterial stock solutions containing bacterial concentrations of 2.07 g_{dry weight}/L (*Bacillus sphaericus*, vegetative cells), 2.15 g_{dry weight}/L (*Bacillus sphaericus*, spores) and 2.56 g_{dry weight}/L (*Pseudomonas stutzeri*). The absorption spectra were measured with a triple-channel fiber optic spectrometer (ST 2000, Ocean Optics). The spectrometer uses a deuterium halogen light source (DH 2000) with a wavelength range from 215 to 1500 nm and a Sony ILX 511 linear CCD-array silicon detector containing 2048 elements. The optical resolution is 2.1 nm (FWHM) due to a slit of 50 µm and a 600-lines grating (blazed at 500 nm) for the visible range (350–1000 nm).

To study the pH dependency of the biosorption, we prepared six samples containing biomass concentrations of 0.38 g_{dry weight}/L and Pu concentrations of 141.7 mg/L in 1.8 mL physiological NaCl-solution at pH values between 1.3 and 6.6. We analyzed the samples by UV/VIS spec-

troscopy before (blanks) and after we added the biomass. After removing the biomass by centrifugation (6000 × g), the remaining plutonium concentration in solution was determined by liquid scintillation counting.

The kinetic studies to determine the time dependency of the biosorption were performed in 5 mL cuvettes using a fixed plutonium concentration of 107.8 mg/L. The pH was adjusted to 4.4 to reduce the amount of the monohydroxo-Pu(VI) complex to less than 10%. Two experimental series were carried out for each strain, using a lower (0.47 g/L for *P. stutzeri* and 0.29 g/L for *B. sphaericus*) and a higher biomass concentration (0.73 g/L for *P. stutzeri* and 0.47 g/L for *B. sphaericus*). In addition, blank solutions without biomass ([Pu] = 107.8 mg/L) were used as controls. After adding the bacteria to the plutonium solution, absorption spectra of the samples were measured at certain time intervals during a 24 hours period using the absorption band at 830 nm of the PuO_2^{2+} ion. After 24 hours, the samples were acidified to pH 0 to remove the plutonium from the biomass. After stirring the solution for one hour, the biomass was separated by centrifugation. Different oxidation states of Pu in the solution were identified by optical absorption spectroscopy. To determine the distribution of oxidation states, 500 µL of the solution were extracted at a time with 500 µL of 0.5 M thenoyltrifluoroacetone (TTA) in xylene and 500 µL di(2-ethylhexyl)phosphoric acid (HDEHP) in toluene. The extractions were performed according to [7]. The Pu concentrations of all phases were measured by liquid scintillation counting.

In a long-term study, we extracted one sample containing 107.9 mg Pu and 0.47 g/L of *Pseudomonas stutzeri* after one month. After determining the total concentration of the reduction products, we separated the biomass from the supernatant by centrifugation. The plutonium on the biomass was removed with 1 M HCl and the plutonium of both fractions was separately extracted with TTA and HDEHP at pH 0.

Results

Sorption and desorption studies

Sorption of plutonium(VI) for vegetative cells of *P. stutzeri* and *B. sphaericus*, and spores of *B. sphaericus* as a function of biomass at pH 5 is presented in Fig. 1. For a better comparison, the results were normalized to the dry weight of the bacteria. The relative biosorption efficiency decreased with increasing biomass concentrations. This is due to an increased agglomeration of the bacteria at higher biomass concentrations, which was observed by microscopy. The spores of *B. sphaericus* showed a much higher biosorption compared to the vegetative cells at low biomass concentration. Biosorption decreased significantly with increasing amounts of biomass. The vegetative cells of *P. stutzeri* and *B. sphaericus* displayed a very similar sorption behavior. They also showed a decreasing biosorption efficiency with increasing amounts of biomass, but the decrease was less than for the *B. sphaericus* spores. No significant differences were observed between the sorption behavior of the vegetative cells of the 24- and the 27-hour culture of *B. sphaericus*. At higher biomass concentrations (> 0.7 g/L), the vege-

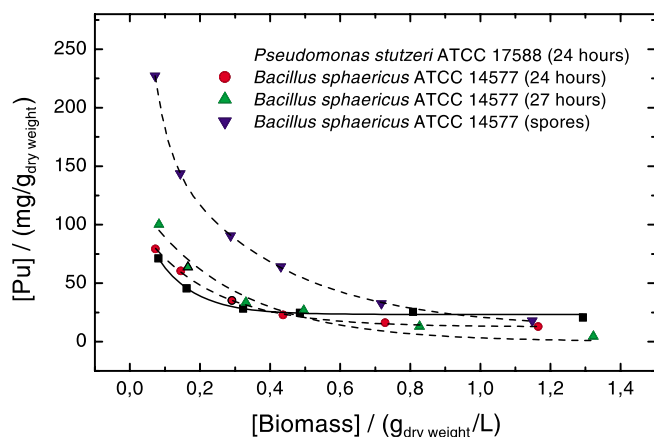


Fig. 1. Plutonium(VI) biosorption of *Pseudomonas stutzeri* ATCC 17588 and vegetative cells and spores of *Bacillus sphaericus* ATCC 14577 at pH 5 as a function of the biomass concentration. The results are normalized to the dry weight of the bacteria.

tative cells of both strains and the spores of *B. sphaericus* showed comparable sorption efficiencies. These results have shown that it is very important to examine the sorption behavior of different strains at various biomass concentrations because different strains can display different sorption behavior with increasing or decreasing biomass concentrations.

To obtain information on the reversibility and the binding strength of the bacterial Pu complexes, we extracted the cell-bound Pu with 0.01 M EDTA solution (pH 5). The percentage of the extractable Pu at different biomass concentrations is shown in Fig. 2. For all biomass concentrations, between 80 and 95% of the plutonium was released from the cells. We observed no significant differences of extraction behavior between the different strains or between the vegetative cells and the spores. These results show that the process is reversible and confirms the formation of surface complexes with functional groups of the cell surface. The bacterial complexes are less stable than the plutonium EDTA-complex. These results are in excellent agreement with the results of our earlier studies on the interaction of U(VI) with different *Bacillus* strains [8].

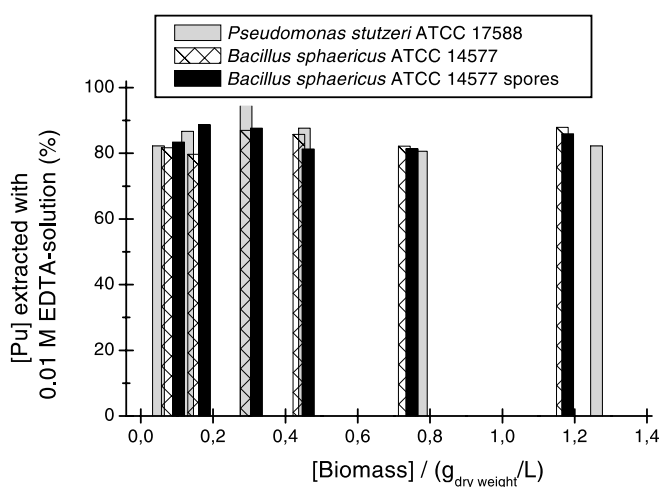


Fig. 2. Percentage of the cell bound Pu extracted from the biomass with 0.01 M EDTA solution (pH 5.0).

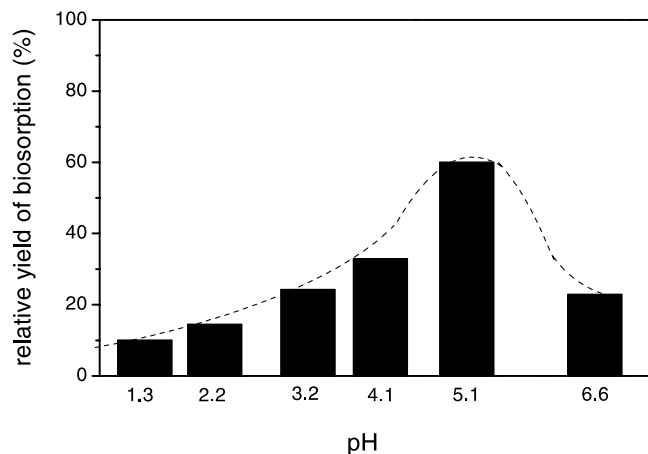


Fig. 3. Total amount of plutonium removed from the solution by *Bacillus sphaericus* ([biomass] = 0.38 g_{dry weight}/L) as a function of the pH.

pH dependency

The amount of Pu(VI) removed by *Bacillus sphaericus* as a function of the pH is presented in Fig. 3. Very little biosorption was observed at low pH values. The amount of Pu bound to the biomass increased with increasing pH according to an increasing deprotonation of the complexing groups on the cell surface. In agreement to the extraction studies with EDTA, this is further proof that the Pu is bound to the bacterial cells via surface complexation. The maximum biosorption was observed at pH 5.1 and decreased again with further increase of the pH. For pH values higher than 4.5, the monohydroxo complex has a significant influence on the species distribution of Pu(VI). The formation of this very strong complex affects also the biosorption and leads to a decrease of the total amount of Pu bound to the biomass with increasing pH.

Influence of the hydrolysis

In order to obtain information on the influence of the hydrolysis on the biosorption process, we studied the biosorption as a function of the ratio of $\text{PuO}_2^{2+}/\text{PuO}_2(\text{OH})^+$ at various pH values between 4.7 to 6.6. The species distribution and the decrease of both species after adding the biomass was determined spectroscopically by analyzing the optical absorption spectra in the region between 825 and 850 nm using the areas of the absorption bands. The plutonium reference solutions without biomass at pH 4.7, 5.2 and 6.6 contained 88.1%, 63.2% and 5.8% of the plutonium aquo ion, respectively. We determined an average stability constant of $\log K = -5.39 \pm 0.13$ for the $\text{PuO}_2(\text{OH})^+$ complex. Our stability constant compares within the error interval to the data of Pashalidis *et al.* [9] ($\log K = -5.68$) and Okajima *et al.* [10] ($\log K = -5.2$) also determined spectroscopically, but is lower than other constants obtained by solubility experiments ($\log K = -5.52$ [11]) or potentiometric titration ($\log K = -5.71$ [12], $\log K = -5.97$ [13]). The decrease of $\text{Pu(VI)}_{\text{aq}}$ and $\text{PuO}_2(\text{OH})^+$ in solution after adding increasing amounts of *Pseudomonas stutzeri* is shown in Fig. 4. The vegetative cells and the spores of *Bacillus sphaericus* showed similar results. For all strains

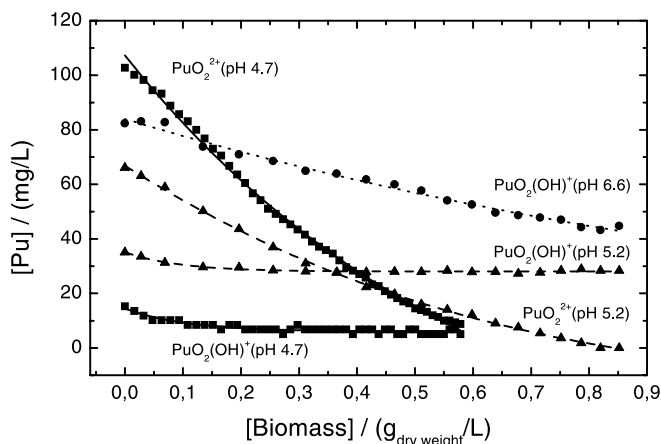


Fig. 4. Decrease of the $\text{Pu(VI)}_{\text{aq}}$ and $\text{PuO}_2(\text{OH})^+$ in solution at pH 4.7, 5.2, and 6.6 after addition of increasing amounts of *Pseudomonas stutzeri*. The concentrations of the Pu species were determined by absorption spectroscopy.

used, the $\text{Pu(VI)}_{\text{aq}}$ ion was quantitatively removed from the solution at all pH values while the concentration of the $\text{PuO}_2(\text{OH})^+$ did not change significantly. We observed a maximum uptake of the plutonium monohydroxo complex of $33.3 \pm 0.8\%$ for *Pseudomonas stutzeri* at pH 5.2, $35.3 \pm 0.9\%$ for *Pseudomonas stutzeri* at pH 6.6, $32.3 \pm 0.7\%$ for *Bacillus sphaericus* vegetative cells at pH 5.1, $32.2 \pm 0.7\%$ for *Bacillus sphaericus* vegetative cells at pH 6.6, and $38.2 \pm 1.1\%$ for *Bacillus sphaericus* spores at pH 4.9. These results indicate that the bacteria preferentially form complexes with the plutonyl aquo ion. The plutonium monohydroxo complex is a very strong complex and only one third of the $\text{PuO}_2(\text{OH})^+$ complexes interacted with the biomass.

Kinetic studies

Fig. 5 shows the decrease of the Pu(VI) concentration in solution with increasing contact time at various biomass concentrations. The main transformation of Pu(VI) occurred during the first 10 minutes. Only a slight additional decrease of Pu(VI) was observed for contact times beyond two hours. The total yield of the plutonium removed from the solution after 24 hours was $68.1 \pm 1.4\%$ and $92.2 \pm 2.1\%$ for *Pseudomonas stutzeri* and $70.8 \pm 1.8\%$ and $88.0 \pm 2.0\%$ for *Bacillus sphaericus* at the lower and higher biomass concentration, respectively (see Fig. 5). It was not possible to fit the time dependency to a mono-exponential law. Fitting the data to a bi-exponential law ($y = y_0 + A_1 e^{-(x/t_1)} + A_2 e^{-(x/t_2)}$)

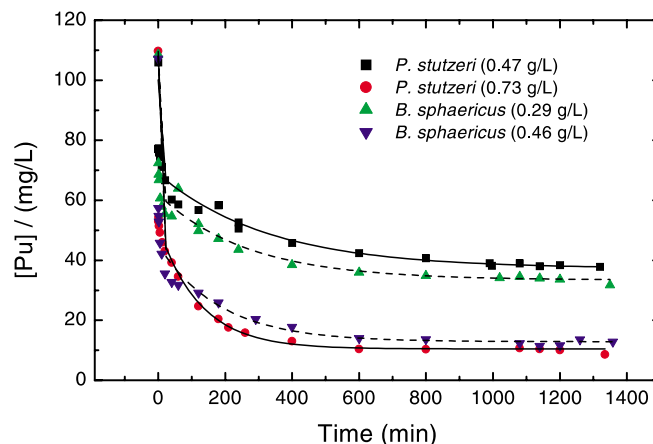


Fig. 5. Decrease of the Pu(VI) concentration ($[\text{Pu}]_{\text{initial}} = 107.8 \text{ mg/L}$) in solution in dependency of the contact time using 0.47 and 0.73 g/L of *Pseudomonas stutzeri* and 0.25 and 0.46 g/L of *Bacillus sphaericus*.

leads to the conclusion that at least two different processes occur after adding the biomass. The fit further provided the contribution of both processes (A_1 and A_2) to the total decrease of the initial Pu(VI) and the amount of Pu(VI) which remained in solution (y_0). The fitting parameters are listed in Table 1. We expect that the complexation of Pu(VI) with functional groups of the biomass is a very fast process. This process can be attributed to the first reaction with the shorter half-life and explains the fast decrease of Pu(VI) in the solution during the first few minutes. The second process is much slower than the first process. The long half-lives indicate that the second process might include a change of the oxidation state.

Determination of the oxidation states of Pu

We used two different methods to identify and quantify different oxidation states of Pu, optical absorption spectroscopy and solvent extraction techniques, to determine if a part of the Pu(VI) is reduced by soil bacteria. Different oxidation states of plutonium have characteristic absorption bands that can be used to identify certain oxidation states [14]. The absorption spectrum of plutonium after 24 h contact with the biomass after redissolution of the Pu on the biomass by acidification to pH 0 is shown in Fig. 6. We identified characteristic absorption bands of Pu(VI) and Pu(V) according to the wavelength and the band shape [14]. The spectrum did not contain any Pu(IV) absorption bands. We conclude from that

Table 1. Fitting parameter of the kinetic data.

Sample	Fitting function: $y = y_0 + A_1 e^{-(x/t_1)} + A_2 e^{-(x/t_2)}$				
	y_0	A_1	t_1	A_2	t_2
<i>Pseudomonas stutzeri</i> : 0.47 g/L	37.8	34.4	0.42	33.3	278.0
<i>Pseudomonas stutzeri</i> : 0.73 g/L	10.4	58.8	0.19	40.4	126.6
<i>Bacillus sphaericus</i> : 0.29 g/L	33.3	46.0	0.37	28.9	270.4
<i>Bacillus sphaericus</i> : 0.46 g/L	12.8	61.8	0.37	32.1	185.0

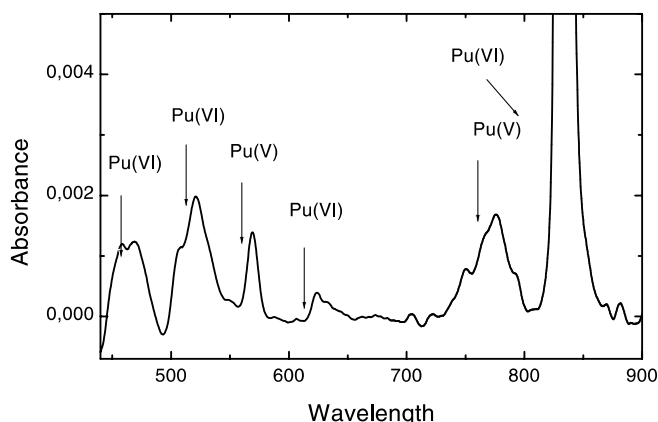


Fig. 6. Absorption spectrum of Pu ($[Pu(VI)]_{\text{initial}} = 107.8 \text{ mg/L}$) at pH 0 after a contact time of 24 hours with $0.73 \text{ g}_{\text{dry weight}}/\text{L}$ of *Pseudomonas stutzeri*.

observation that the initially added Pu(VI) is partly reduced to Pu(V) by interaction with the bacteria.

Because of the low absorption coefficients of Pu(IV) and Pu(V) [15] and the low concentration of Pu in the solution, the quantification of the different Pu-species was performed by solvent extraction. Solvent extraction with TTA and HDEHP allows a selective separation of different oxidation states of Pu at low concentration levels between 10^{-6} and 10^{-10} mol/L , which is below the sensitivity range of the absorption spectroscopy [7]. The results of the solvent extractions of the four samples used for the kinetic studies are listed in Table 2. After 24 hours, the samples contained three different species: $Pu(VI)_{\text{aq}}$ in the solution, $Pu(VI)$ bound to the biomass and $Pu(V)$. In agreement with the spectroscopic results, no Pu(IV) was formed during 24 hours. Whereas the ratio of $Pu(VI)_{\text{biomass}}/Pu(VI)_{\text{aq}}$ increased with increasing biomass concentration, the four samples contained almost the same amount of $Pu(V)$. Comparing the distribution of the oxidation states after 24 hours (Table 2) with the fitting parameter of the kinetic studies (Table 1), we found a very good agreement between A_1 and $Pu(VI)_{\text{biomass}}$, A_2 and $Pu(V)$ and y_0 and $Pu(VI)_{\text{solution}}$. These results prove that the reaction with the fast kinetic (A_1 , t_1) can be attributed to the complexation of Pu(VI) with the biomass, whereas the slow process (A_2 , t_2) describes the reduction of Pu(VI) to Pu(V).

Table 2. Results of the solvent extraction with 0.5 M TTA and 0.5 M HDEHP after a contact time of 24 hours (error: $< 5\%$).

Biomass (mol/L)	Pu(IV) mg/L (%)	Pu(V) mg/L (%)	Pu(VI) _{solution} mg/L (%)	Pu(VI) _{biomass} mg/L (%)
<i>Pseudomonas stutzeri</i> : 0.47 g/L	–	28.6(27.0)	33.8(31.9)	43.5(41.1)
<i>Pseudomonas stutzeri</i> : 0.73 g/L	–	34.9(31.8)	8.6(7.8)	66.2(60.3)
<i>Bacillus sphaericus</i> : 0.29 g/L	–	34.0(31.3)	31.7(29.2)	42.8(39.4)
<i>Bacillus sphaericus</i> : 0.46 g/L	–	36.4(34.0)	12.8(11.9)	58.0(54.1)

Table 3. Results of the solvent extraction of the supernatant and the biomass with 0.5 M TTA and 0.5 M HDEHP after a contact time of one month (error: $< 5\%$).

Pu(IV) mg/L (%)		Pu(V) mg/L (%)		Pu(VI) mg/L (%)	
17.4(16.1)		46.8(43.4)		43.7(40.5)	
Supernatant mg/L (%)	Biomass mg/L (%)	Supernatant mg/L (%)	Biomass mg/L (%)	Supernatant mg/L (%)	Biomass mg/L (%)
1.6(1.5)	15.7(14.6)	40.4(37.4)	6.6(6.1)	19.4(18.0)	24.2(22.4)

To determine if also Pu(IV) will be formed after a long contact time, we performed long term studies after a contact time of one month using *P. stutzeri*. The results are given in Table 3. The comparison of these results with the oxidation state distribution after 24 hours shows that the percentage of Pu(V) is almost 10% higher. In addition, 16.1% Pu(IV) was formed.

After determining the total concentration of the reduction products, we extracted the Pu on the biomass and in the supernatant separately to obtain information whether the reduction products are localized in the solution or bound to the cells. The species distributions of both fractions (also given in Table 3) have shown that more than 90% of the Pu(IV) was removed together with the biomass, whereas the main part of the Pu(V) was found in the supernatant due to the weak complexation properties of the Pu(V) ion. Furthermore, both fractions contained similar amounts of Pu(VI).

Discussion

The present results have demonstrated that there is a strong interaction of hexavalent plutonium with aerobic soil bacteria like *Bacillus sphaericus* and *Pseudomonas stutzeri*. On the basis of our results, we developed a model which describes the overall process (see Fig. 7). Contrary to the interaction of U(VI) with aerobic soil bacteria, the interaction of Pu(VI) with *Bacillus sphaericus* and *Pseudomonas stutzeri* includes three processes which have different time scales:

In a first step, the Pu(VI) is bound to the biomass. This process is characterized by very fast kinetics and depends strongly on the amount of biomass used which determines the amount of available binding sites of the cells. Extraction studies and sorption experiments at different pH values have shown that surface complexes with functional groups on the cell surface are formed. Former results on the complexation of U(VI) with *Bacillus* strains have shown that the uranium is coordinated with phosphate groups [8, 16]. The analysis of EXAFS spectra of the Pu(VI) complex with *B. sphaericus* provided information on the coordination and bond lengths ($Pu-O_{\text{axial}}$: 1.78 Å, $Pu-O_{\text{equatorial}}$: 2.42 Å and $Pu-P$: 3.70 Å) and confirmed that the plutonium is also bound to organophosphate groups [17].

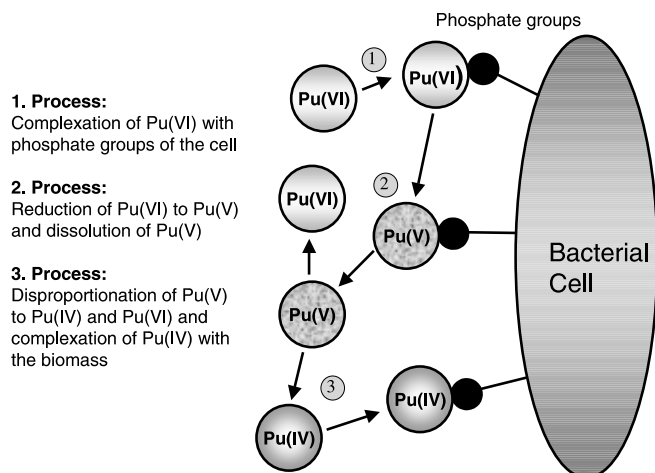


Fig. 7. Illustration of the different processes of the interaction of Pu(VI) with aerobic soil bacteria.

In a second step, a part of the cell-bound Pu(VI) is reduced to Pu(V) by interaction with the biomass. This is a much slower process compared to the binding of Pu(VI) to the biomass. Only one third of the initial Pu(VI) was reduced after 24 hours. Due to the weak complexation ability of the PuO_2^{2+} ion, more than 85% of the Pu(V) was found in solution.

The comparison with the blank solutions without biomass has shown, that the plutonium reduction is caused by interaction with the bacteria. Thereby, two different processes can play a role. In the non-metabolic process the plutonium reduction is a result of an oxidation of the organic matter or functional groups on the cell surface. Because we used living cells, also a metabolic process as described in [18] may be involved. Microorganisms obtain energy for growth by catalyzing oxidation and reduction reactions. Comparing the redox potentials of the $\text{O}_2/\text{H}_2\text{O}$ couple with that of the $\text{PuO}_2^{2+}/\text{PuO}_2^+$ couple, aerobic soil bacteria should be able to reduce Pu(VI) to Pu(V) concurrently with oxygen depletion [18]. Due to the fact that our experiments were performed in NaCl solution without providing any further nutrients, this process is probably of minor importance.

Our long term studies have shown that after a contact time of one month 16.1% Pu(IV) was formed (third process). Considering the living conditions of the bacteria, we expect that the main part of the bacteria died before the end of the long term experiment because of a lack of nutrients. This means that the metabolic activities of the biomass should decrease with increasing contact time. It is more likely that under our experimental conditions the Pu(IV) was produced by disproportionation of Pu(V) and/or autoredox of Pu(VI). The very slow kinetics of the Pu(IV) formation, observed in our experiments, are in good agreement with literature data for both processes under comparable conditions [15, 19–21].

Our results have shown that the interaction of plutonium with aerobic soil bacteria causes changes of the oxidation state which have an important impact on the migration behavior. The reduction of the Pu(VI) bound to the biomass to Pu(V) leads to an increased dissolution of the cell bound

plutonium. On the other hand, the formation of Pu(IV) increases the immobility of plutonium. This information is very important for predictions on the migration of actinides in natural systems as well as for remediation purposes.

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References

- Choppin, G. R., *Radiochim. Acta*, **43**, 82 (1988).
- Wildung, R. E., Garland, T. R.: The Relationship of Microbial Processes to the Fate and Behavior of Transuranic Elements in Soils, Plants, and Animals. In: *Transuranic Elements in the Environment*. (Hanson, W. C., Ed.), DOE/TIC-22800, Technical Information Center/US Department of Energy, Washington, DC (1980), p. 300.
- Francis, A. J., Gillow, J. B., Dodge, C. J., Dunn, M., Mantione, K., Strietelmeier, B. A., Pansoy-Hjelvik, M. E., Papenguth, H. W.: *Radiochim. Acta* **82**, 347 (1998).
- Strietelmeier, B. A., Sebring, R. J., Gillow, J. B., Dodge, C. J., Pansoy-Hjelvik, M. E., Kraus, S. M., Leonard, P. A., Triay, I. R., Francis, A. J., Papenguth, H. W.: Plutonium Interaction with a Bacterial Strain isolated from the Waste Isolation Pilot Plant (WIPP) Environment. American Chemical Society, Division of Environmental Chemistry Preprints of Extended Abstracts Vol. **36** (2) (1996).
- Rusin, P. A., Quintana, L., Brainard, J. R., Strietelmeier, B. A., Tait, C. D., Ekberg, S. A., Palmer, P. D., Newton, T. W., Clark, D. L.: *Environ. Sci. Technol.* **28**, 1686 (1994).
- AlMahamid, I., Becraft, K. A., Hakem, N. L., Gatti, R. C., Nitsche, H.: *Radiochim. Acta* **74**, 129 (1996).
- Nitsche, H., Lee, C., Gatti, R. C.: *J. Radioanal. Nucl. Chem. Articles* **124/1**, 171 (1988).
- Panak, P. J., Raff, J., Selenska-Pobell, S., Geipel, G., Bernhard, G., Nitsche, H.: *Radiochim. Acta* **88**, 71 (2000).
- Pashalidis, I., Kim, J. I., Ashida, T., Grenthe, I.: *Radiochim. Acta* **68**, 99 (1995).
- Okajima, S., Reed, D. T., Beitz, J. V., Sabau, C. A., Bowers, D. L.: *Radiochim. Acta* **52/53**, 111 (1991).
- Kim, J. I., Bernkopf, M., Lierse, Ch., Koppold, F.: In: *Geochemical Behaviour of Disposed Radioactive Waste*. (Barney, G. S., Navratil, J. D., Schulz, W. W., Eds.), ACS Symposium Series 246, Chapt. 7 (1984).
- Kraus, K. A., Dam, J. R.: *Natl. Nucl. Energy Ser. IV* **14B**, 528 (1949).
- Cassol, A., Magon, L., Portanova, R., Tondello, E.: *Radiochim. Acta* **17**, 28 (1972).
- Cohen, D.: *J. Inorg. Nucl. Chem.* **18**, 211 (1961).
- Keller, C.: *The Chemistry of the Transuranium Elements*. Verlag Chemie GmbH, Weinheim (1971), p. 426.
- Henning, C., Panak, P., Reich, T., Roßberg, A., Selenska-Pobell, S., Bernhard, G., Nitsche, H.: *Institut für Radiochemie, Forschungszentrum Rossendorf, Report-FZR* **272**, 88 (1999).
- Panak, P. J., Booth, C. H., Shuh, D. K., Nitsche, H.: X-ray Absorption Spectroscopy of Plutonium Complexes with *Bacillus sphaericus*, in preparation.
- Basnuszak, J. E., Rittmann, B. E., Reed, D. T.: *J. Radioanal. Nucl. Chem.* **241/2**, 385 (1999).
- Cleveland, J. M.: *The Chemistry of Plutonium*. American Nuclear Society, La Grange Park (1979), p. 26.
- Rabideau, S. W.: *J. Am. Chem. Soc.* **79**, 6350 (1957).
- Gevantman, L. H., Kraus, K. A.: Chemistry of Plutonium(V). Stability and Spectrophotometry. In: *The Transuranium Elements*. (Seaborg, G. T., Katz, J. J., Manning, W. M., Eds.) IV-14B, McGraw Hill, New York (1949), p. 241.